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AN ATTEMPT FOR EXPERIMENTAL INDUCTION OF EPIDEMIC HEPATITIS INFECTIOUS PROCESS. FIRST REPORT*

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Epidemic hepatitis, particularly in the period following the second world war when a pandemic widespread took place, assumed a paramount importance as a problem concerned with public health on world-wide scale. On account of the failure to isolate the chief factor causing the disease and limited experimentation on volunteers, the production of an experimental model on a test animal appears to be of utmost significance for the comprehensive study of the various aspects of the affection. Research work in this field is very intense (5, 6, 8, 10). Pyatkin (7) states that animals are hardly susceptible to infection by the epidemic hepatitis virus. A number of authors (5, 9, 10) however, establish an asymptomatic course of the disease, discovering intranuclear involvements in hepatic cells of contaminated experimental animals.

Proceeding from the data reported, the authors make an attempt to provoke and infections process of epidemic hepatitis in white albino rats, tracing up the changes within the nuclei of the liver cells by means of the Feulgen's test.

Material and method

Fifty six white albino rats were employed, weighing from 200—220 gr. Contamination was performed by intramuscular inoculation: 40 — with 0.5 cc serum obtained from a patient(+) on the second post-icterus day, and 16 — left intact for control purposes. At fixed time-intervals, 3 contaminated and 2 control rats were sacrificed. The livers were removed for carrying out the Feulgen's test, using the Carnua fixative. Paraffin sections were prepared, thick 5 microns. Through acid hydrolysis (the binding between pyrimidine bases and desoxyribose is disrupted) aldehyde groups were formed, subjected to the effect of the Schif reactive. An end-compound was obtained tinged violet-reddish. (**)

One hundred nuclei were counted in each preparation and determination was carried out of the following indices: size and volume of the hepatic nuclei, number and size of nucleoli, structure and intensity of staining of the Feulgen positive material within the nuclei.

(+) *Case report* — Ivan K. S., aged 17, profession — worker. Epidemic hepatitis contact; admitted to the clinic of infectious diseases at the

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Higher Medical Institute — Varna on March 15, 1966 (history of illness No. 409/15. III. 1966) with complaints dating since March 8, 1966, manifested with feebleness, general indisposition, lack of apetite, nausea without vomiting, heaviness sensation in the right subcostal area. On March 12, 1966 his urine became turbid and on March 16 the sclera became yellow. Liver palpated one centimeter beneath the costal arch, painful. Weltmann's serum test — 8 tubes, thymol turbidity test — 63 Ph. u., total bilirubin — 2,5% mgr, transaminase — 550 Ph. u., cholinesterase — 2220. Bilirubin in the urine — positive, urobilinogen — increased. Dismissed on April 9, 1966 with definitive diagnosis epidemic hepatitis.

Results and discussion

The hepatic cells' nuclei in the preparations of the control rats exhibit radiant localization around the central veins, with irregular oval shape (figs. 1-a and 2-a), average diameter 7,14—0,34 microns and average volume 189,6 ± 29,3 cubic microns. The nuclei are distributed in several subgroups depending on the diameter (see diagram No. 1): from 5—6 microns — 27%, from 7 to 8 microns — 63% and from 9 to 10 microns — 10%. The periphery of the nuclei is hardly outlined. Within the nuclei of hepatic cells the reticular structure is detected, displaying fine granulations and comparatively uniform pigmentation. Each nucleus contains several nucleoli, ranging from 2 — 4—5 with diameter 1,5—2 microns.

One week after the contamination the nuclei preserve their radiant disposition, but are adequately rounded and substantially augmented in size (see figs 1-b and 2-b), with average diameter measuring 8,40 ± 0,37 microns and mean volume 309,3 ± 40,3 cubic microns. The data reported are statistically reliable. Nuclear grouping according to diameter is as follows: from 5—6 microns — 5%, from 7 to 8 microns — 45%, from 9 to 10 microns — 48% and from 11 to 12 microns — 2%. The nuclei disclose well manifested peripheral margination, with intranuclear granulations becoming rather rough. The extent of nuclear staining is insignificantly increased. The majority of the nucleoli, whose number in each separate nucleus is diminished, are with a diameter ranging from 2—2,5 microns.

A similar picture is observed on the third post-contamination week.

After the fourth week, the picture is significantly altered. The nuclei (see figs. №№ 1-b and 2-b) exhibit an average diameter measuring 7,35±0,33 microns and average volume 207,6±31,0 cubic microns, with relatively intact radiant disposition around the central veins. The pattern of nuclear grouping is the following: from 5 to 6 microns — 13%, from 7 to 8 microns 83% and from 9 to 10 microns — 4%. Most of the granulations are filled up with gross granulations, displaying an intense colouring. The nucleoli are isolated and consistently enlarged, occupying the central nuclear area.

The picture observed during the fifth week is similar. Thereafter, the nuclei again assume an irregular oval shape, the average diameter is reduced, of the nucleoli as well, and their number in single nuclei begins to increase. The changes described reveal a rather protracted course, up to the 8th week (see figs. 1-d and 2-d) and thereupon a substantial normalization occurs, mostly pronounced about the 3rd month after contamination.

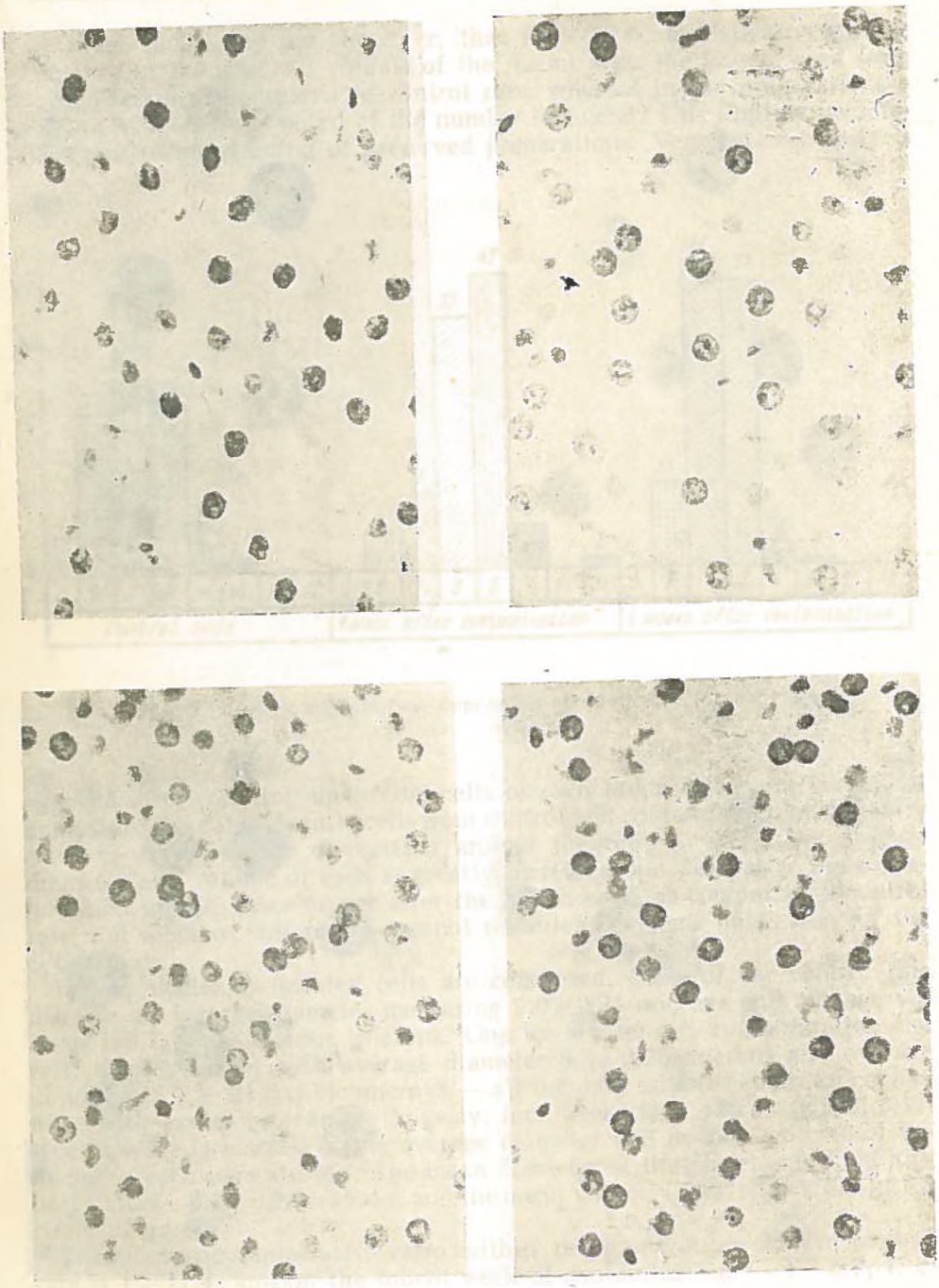

Fig. 1. Hepatic nuclei

a) from control rat, *b*) one week after contamination, *c*) four weeks after contamination, *d*) eight weeks after contamination. Staining after Feulgen. Oc. 10; ob. 40

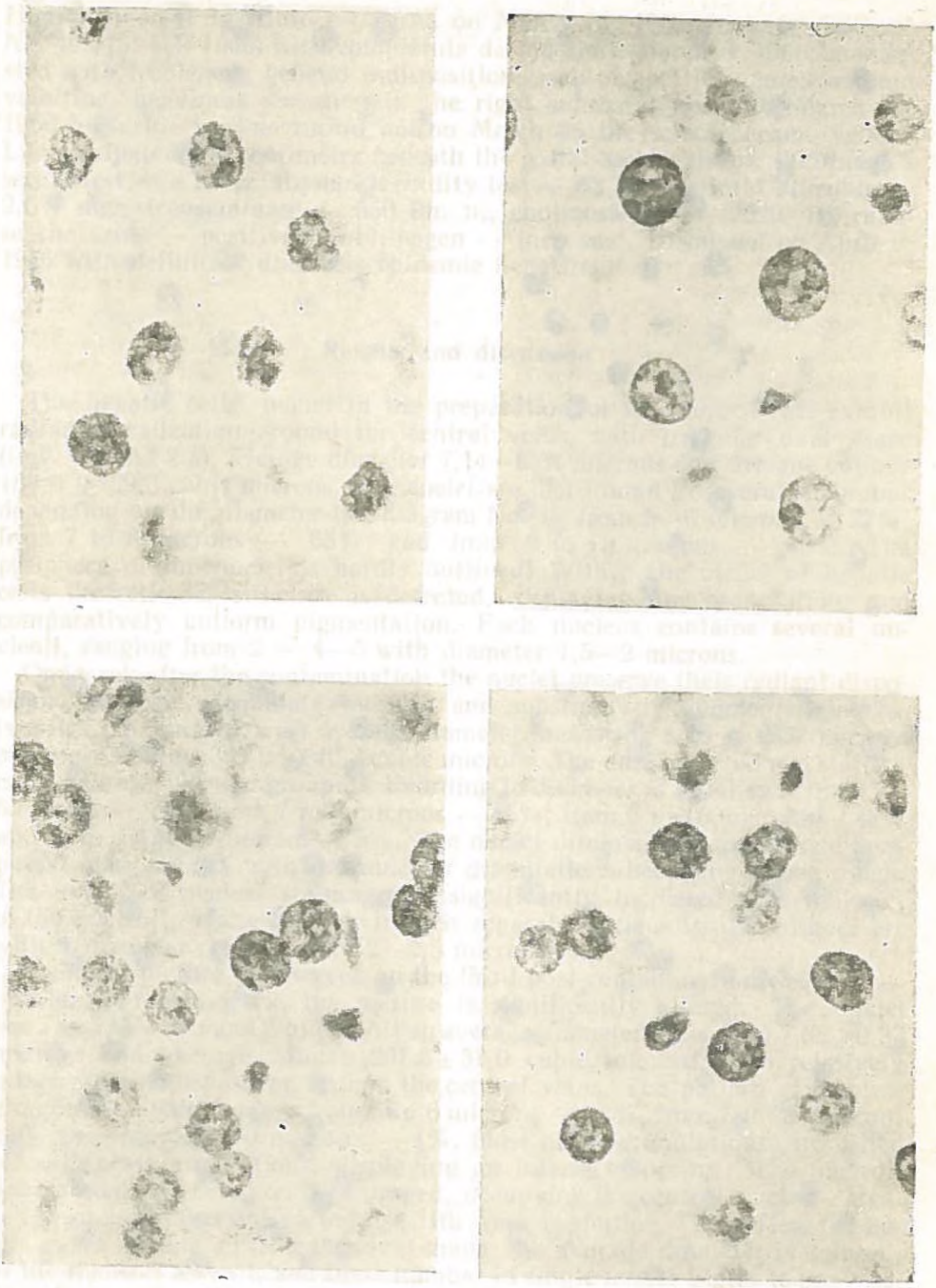

Fig. 2. Hepatic nuclei

a) from control rat, *b*) one week after contamination, *c*) four weeks after contamination, *d*) eight weeks after contamination. Staining after Feulgen. Oc. 10; ob. 100

It must be pointed out, however, that there is no statistically reliable difference in the size and volume of the nuclei after the fourth week from contamination as compared to control rats, whereas in the preparations a substantial increase is noted of the number of nuclei. This implied hematoxylin and eosine staining of preserved preparations. Very interesting facts

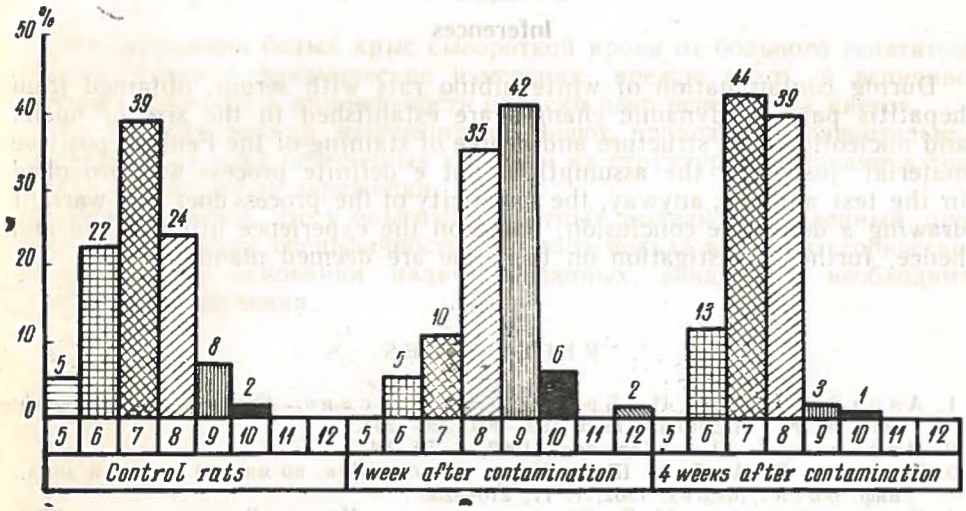

Fig. 3. Nuclear distribution depending on their size (diameter)

emerged after counting up to 100 cells of each preparation and statistical analysis of the data. Hepatic cells from control and contaminated rats equally conserve their radiant disposition around the central veins, the average diameter and volume of cells is greatly increased one week after the contamination and in lesser degree after the fourth week as compared to control rats, but anyhow, differences are not reliable. The same holds true for the cytoplasm.

Insofar nuclei of isolated cells are concerned, those of the control rats disclose an average diameter measuring $7,0 \pm 0,24$ microns and average volume $178,7 \pm 23,7$ cubic microns. One week after the contamination the cells display nuclei with average diameter $8,3 \pm 0,25$ microns and average volume $302,6 \pm 24,3$ cubic microns — all the data established are in conformity with earlier recordings. Anyway, four weeks later, a substantial relative increase is marked of the average diameter and average volume of the nuclei in each separate cell. The mean diameter of the nuclei exhibited highest values—$8,5 \pm 0,23$ microns, and the mean volume likewise — $320,2 \pm 21,8$ cubic microns.

The nuclear-cytoplasmatic ratio within the preparations of the control rats is 1 : 11,4, and on the fourth week of contamination — 1 : 6,7, i. e. anapproximately twofold increase is established.

A similar increase is noted in the average volume of nuclei in the preparations on the fourth week after contamination as compared to those of con-

trol rats, namely 1 : 1,8 (*). Thereupon, the changes conform with those already described.

Contaminated and control white albino rats alike did not show deviations in weight, nutrition and temperature.

Inferences

During contamination of white albino rats with serum, obtained from hepatitis patient, dynamic changes are established in the size of nuclei and nucleoli, in the structure and degree of staining of the Feulgen positive material, justifying the assumption that e definite process was provoked in the test animals; anyway, the specificity of the process does not warrant drawing a definitive conclusion, based on the experience hitherto had and hence, further investigation on the issue are deemed mandatory.

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* According to preliminary experimental data, part of the contaminated alb rats were treated with gamma globulin; on the fourth week, the contaminated but not subjected to gamma globulin treatment animals show a nearly twofold increase of DNA, from 36,1 to 75,6 mgr P per 100 gr fresh weight (according to the methoa of Tzanev and Markov).

ПОПЫТКА ВЫЗВАТЬ ЭКСПЕРИМЕНТАЛЬНЫЙ ИНФЕКЦИОННЫЙ ПРОЦЕСС ЭПИДЕМИЧЕСКОГО ГЕПАТИТА

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РЕЗЮМЕ

При заражении белых крыс сывороткой крови от больного гепатитом, устанавливаются динамические изменения, прежде всего, в величине, объеме, структуре и интенсивности окраски ядер печеночных клеток.

Ко второму месяцу изменения начинают проходить. Сравнительная нормализация ядер печеночных клеток и их структуры наступают к третьему месяцу после заражения.

Предполагается, что у опытных животных вызван определенный процесс, в отношении специфичности которого нельзя взять категорическое отношение на основании наличных данных, ввиду чего необходимы дальнейшие изучения.